

Figure 1

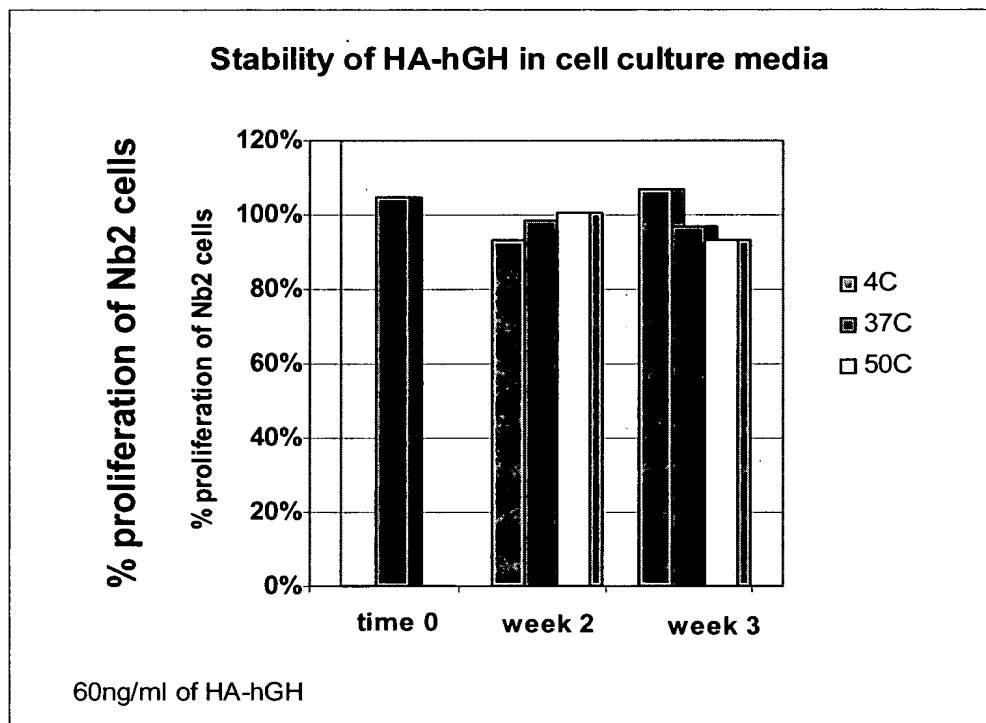
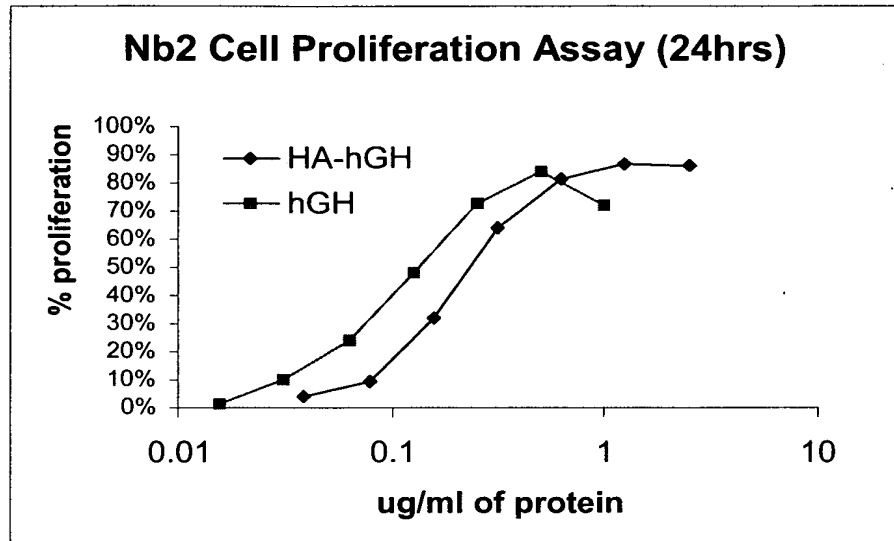
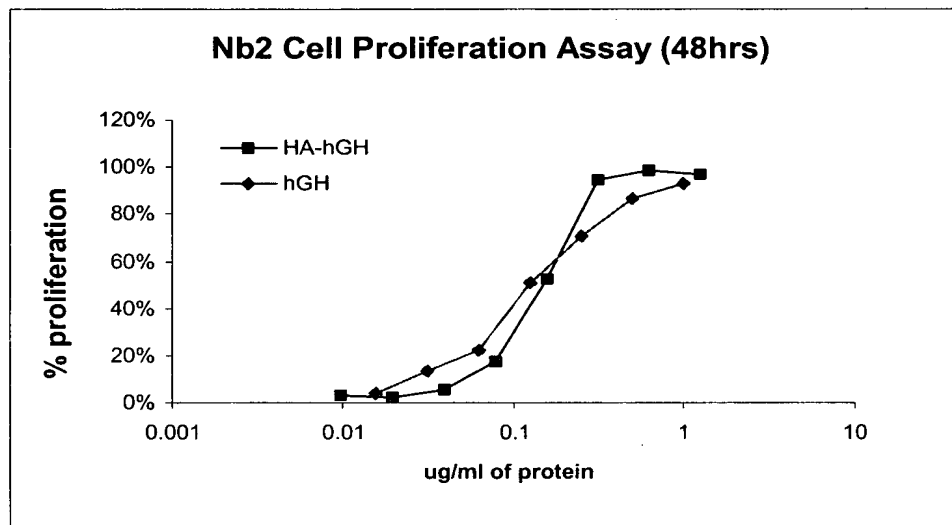


Figure 2

**Figure 3A****Figure 3B**

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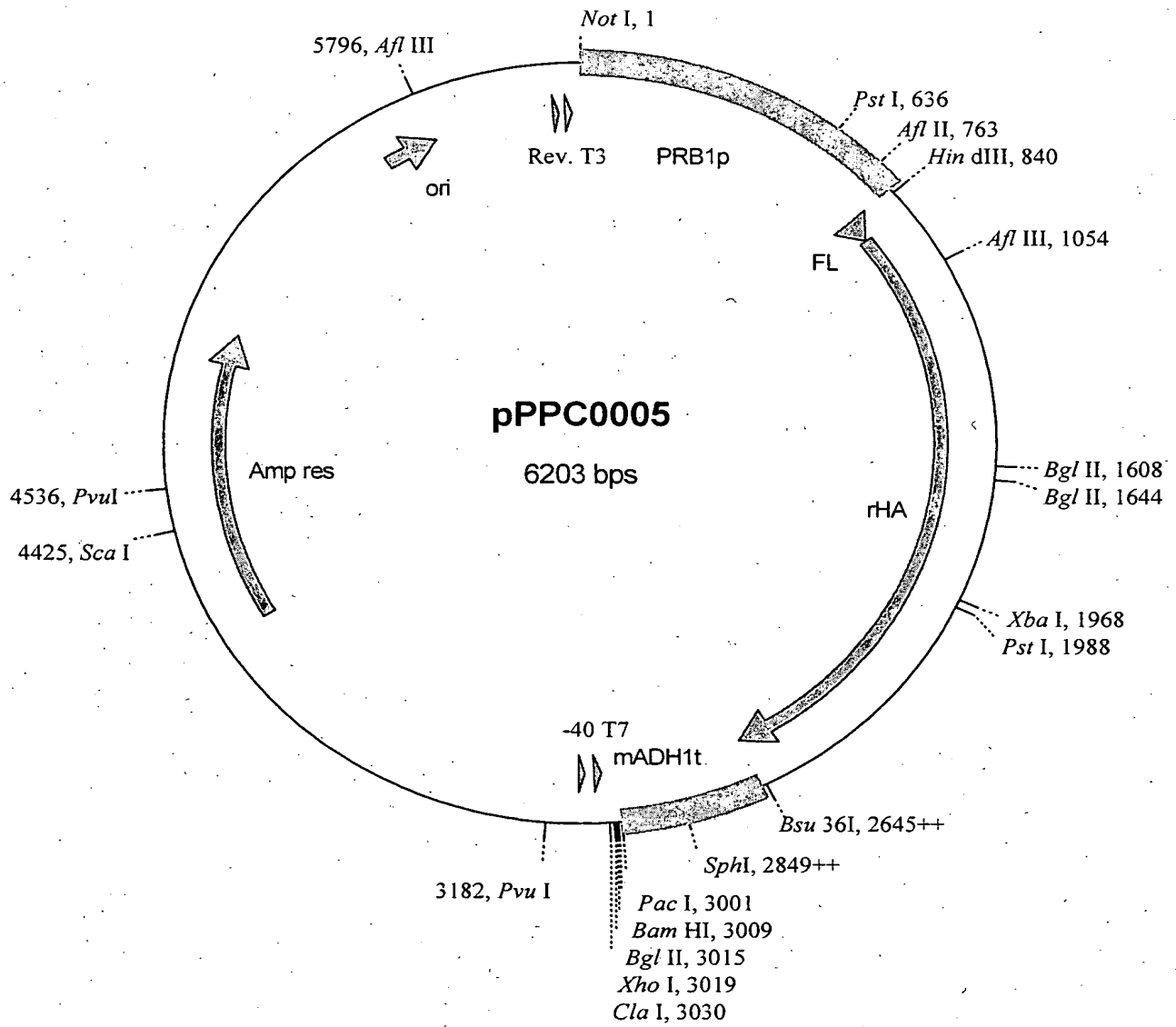


Figure 4

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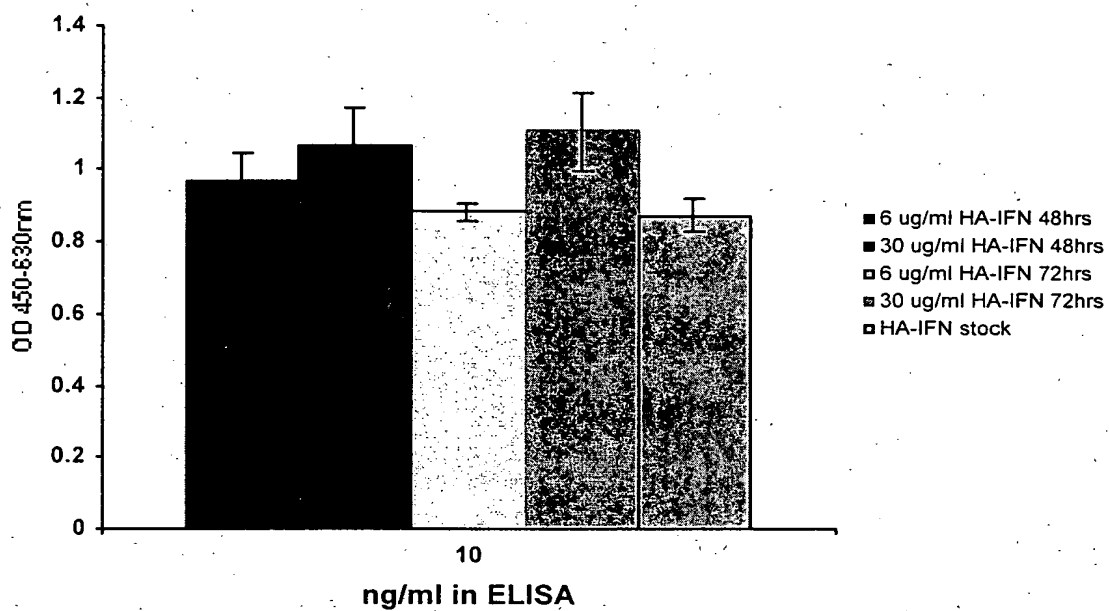


Figure 5

Figure 6

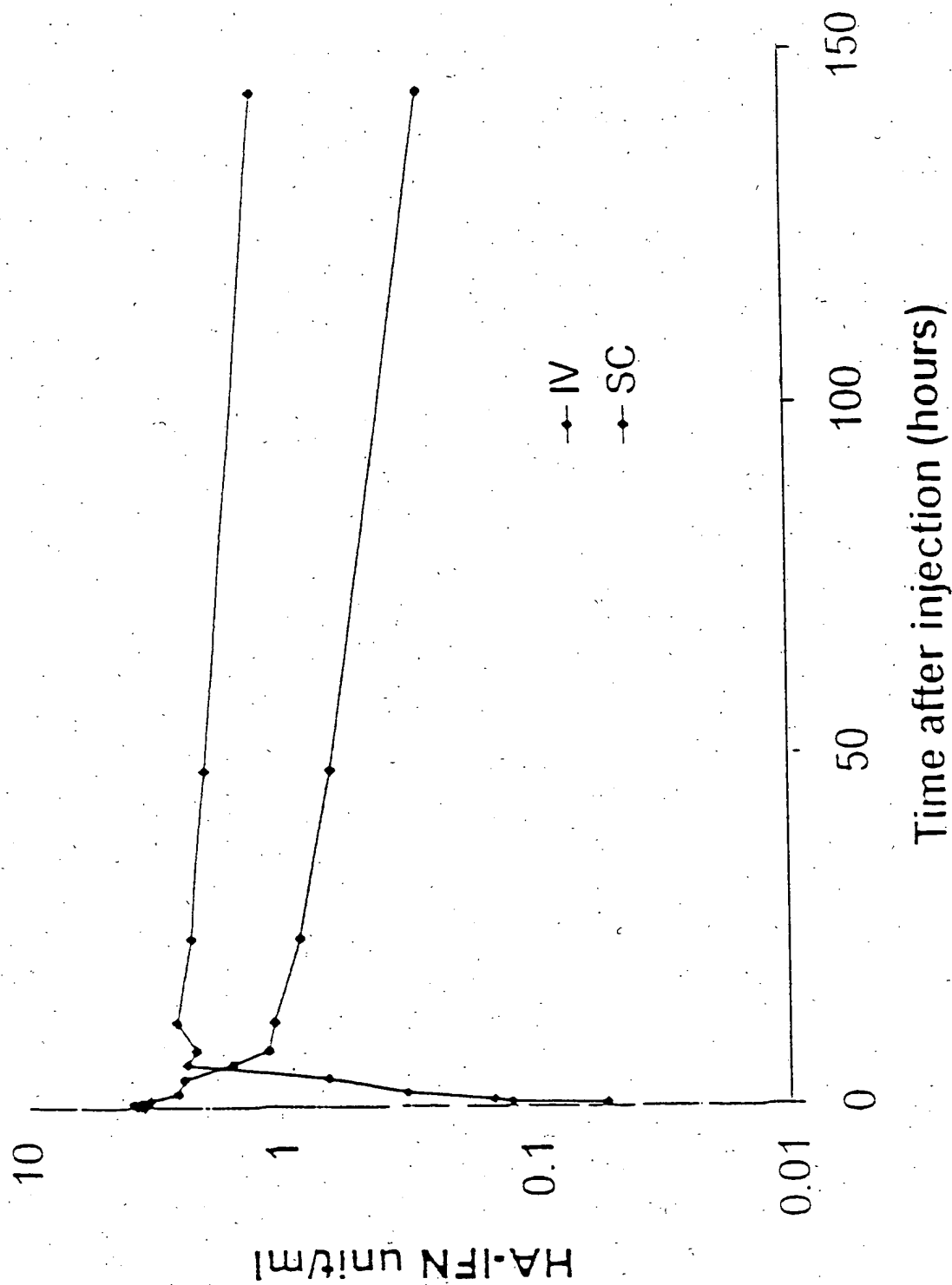
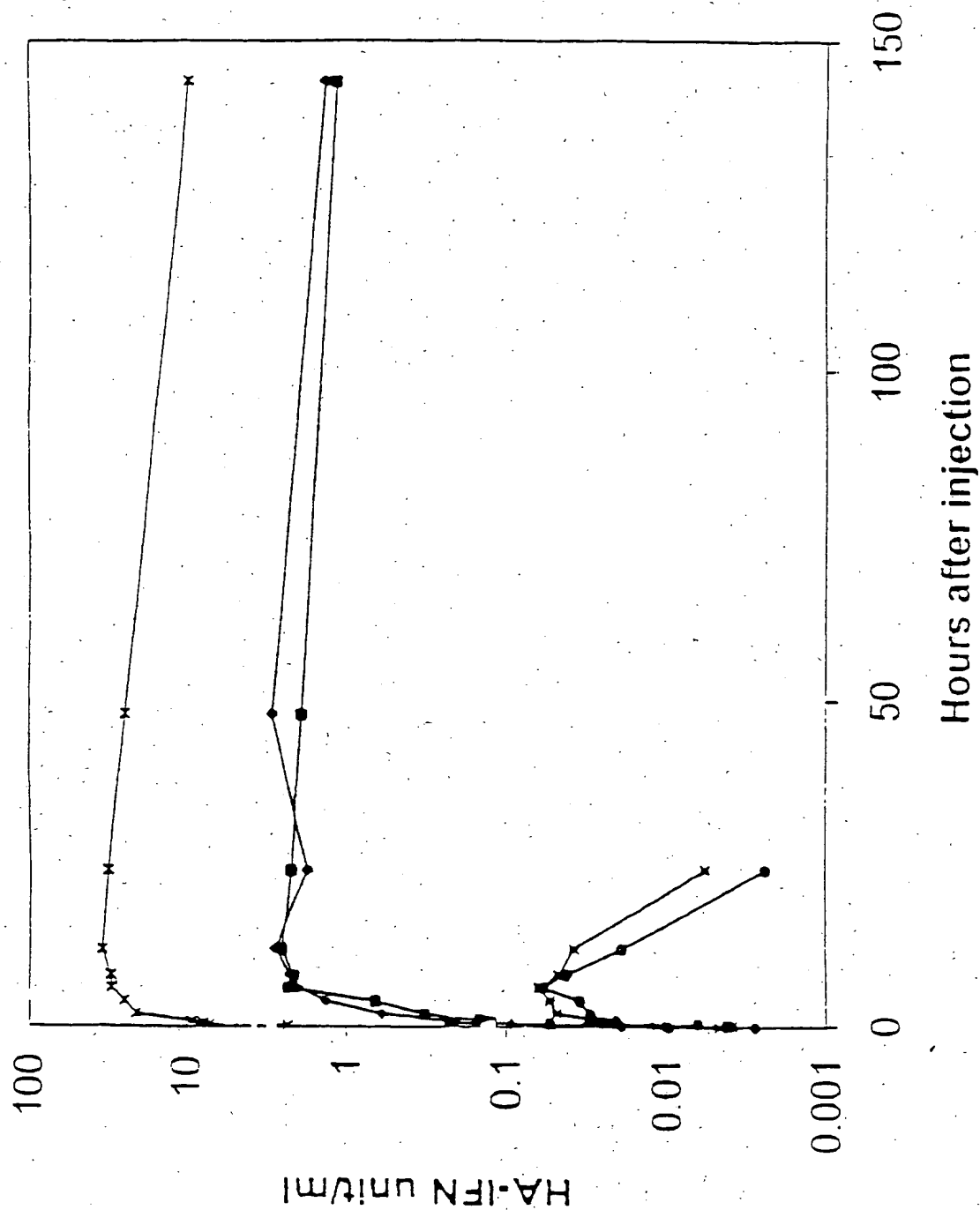


Figure 7



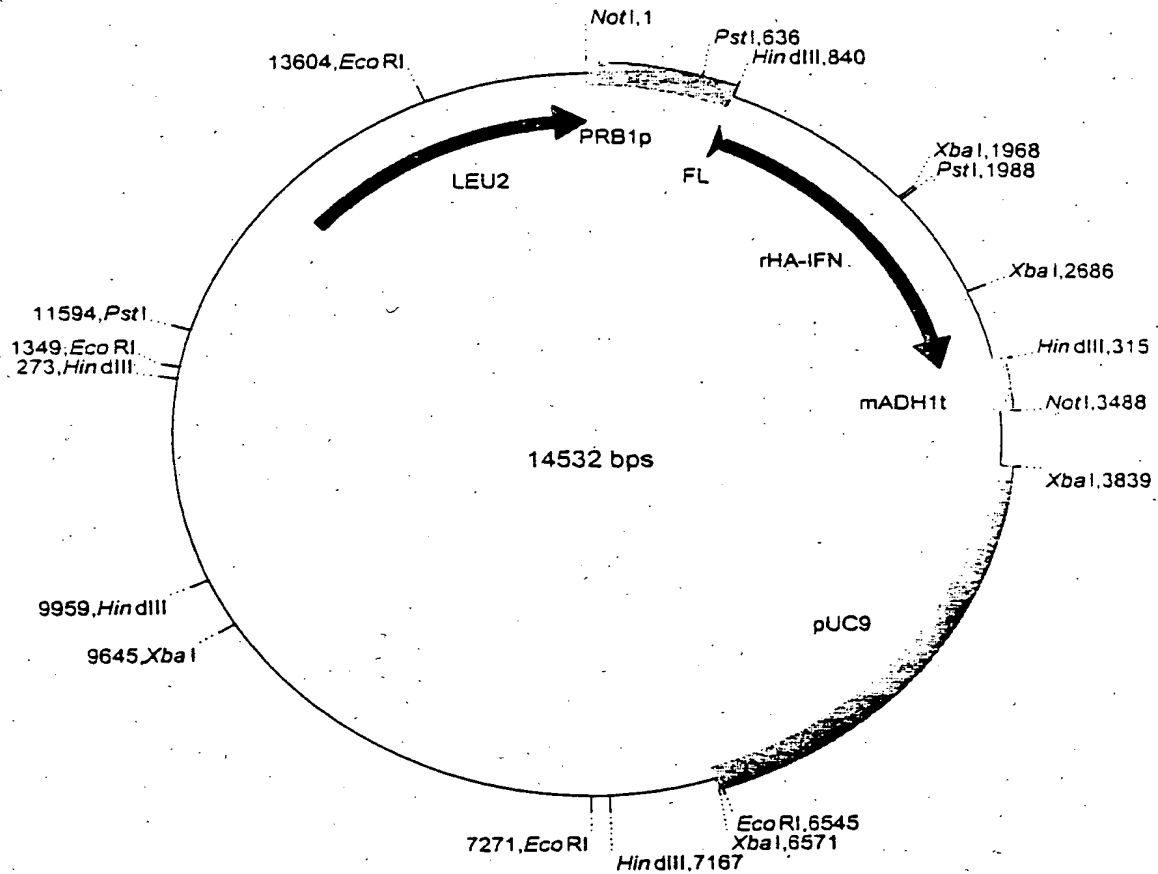


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises:
PRB1 promoter, from *S. cerevisiae*.
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF α -1 leader.
 HA-IFN α coding sequence with a double stop codon (TAATAA)
ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind* III/*Bam*HI fragment generally used.

Figure 8

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Localisation of 'Loops' based on the HA Crystal Structure
which could be used for Mutation/Insertion

```

1  DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
   HHHHH HHH      HHH HHHHHHHHHHH      HHHHH HHHHHHHHHHH

      I                      II                      III
51  KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKOEPERNE
   HHHHH      HHHHH HHHHH      HHHH H      HHHH

101 CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
   HHHH      H      HHHHHHHHH      HHHHHHHHH HHHHH

      IV
151 APELLFFAKR YKAAFTECCO AADKAACLLP KLDEL RDEGK ASSAKQRLKC
   HHHHHHHHHHH HHHHHHHHH      HHHHH HHHEHHHHHH HHHHHHHHHHH

      V
201 ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECHGDL
   HHHHH      HH HHHHHHHHHHH HH      HHH HHHHHHHHHHH HHHHHH      HH

      VI                      VII
251 LECADDRADL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
   HHHHHHHHHHH HHHHH      HHHHH      HHHHHHH H

301 DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLA
   HHHH      HHHHHH      HHHHHHH HHHHHH      HHHHHHHH

      VIII
351 KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
   HHHHHHHHHHH      HH      H      HHHHH HHHHHHHHHHH HHHHHHH

      IX
401 YKFQNALLRV YTKKVPQVST PTLVEVSRNL GKVGSKCCCKH PEAKRMPCAE
   HHHHHHHHHHH HHHH      H HHHHHHHHHHH      HHH      HHHHHHHH

      X                      XI
451 DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPPCFSA LEVDETYVPK
   HHHHHHHHHHH HHHHH      HHHHHHHHH      HHHHHHHH

501 EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
   HHH      HHH HHHHMMHHHH HHH      HHHHHHHH

      XII
551 FAAFVEKCCCK ADDKETCFAE EGKKLVAAASQ AALGL
   HHHHHHHHH      HHHH HHHHHHHHHHH HH

```

Loop	Loop
I Val54-Asn61	VII Glu280-His288
II Thr76-Asp89	VIII Ala362-Glu368
III Ala92-Glu100	IX Lys439-Pro447
IV Gln170-Ala176	X Val462-Lys475
V His247-Glu252	XI Thr478-Pro486
VI Glu266-Glu277	XII Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

IV

```

151  APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHH
  
```

IV

```

151  APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHH
  
```

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

(X)_n



IV

```

151  APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHH
  
```

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

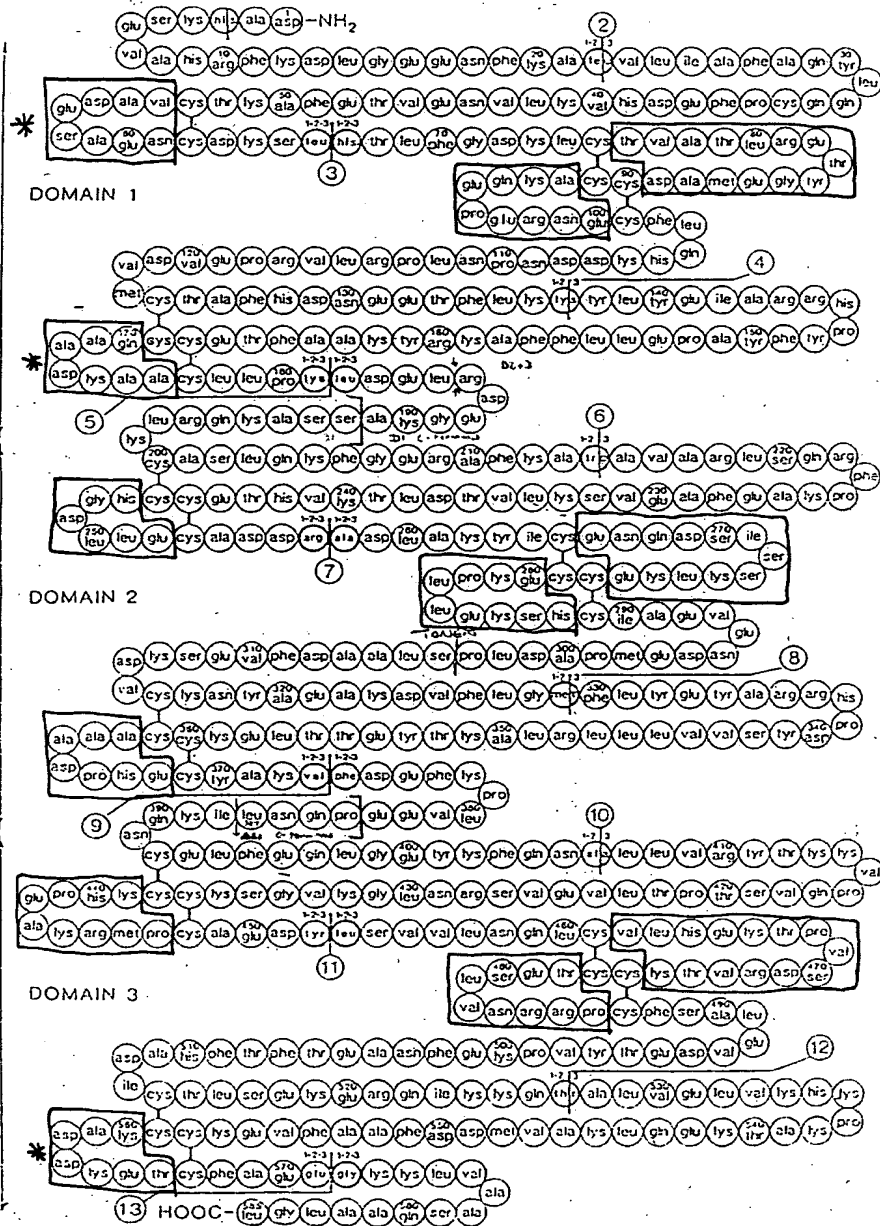
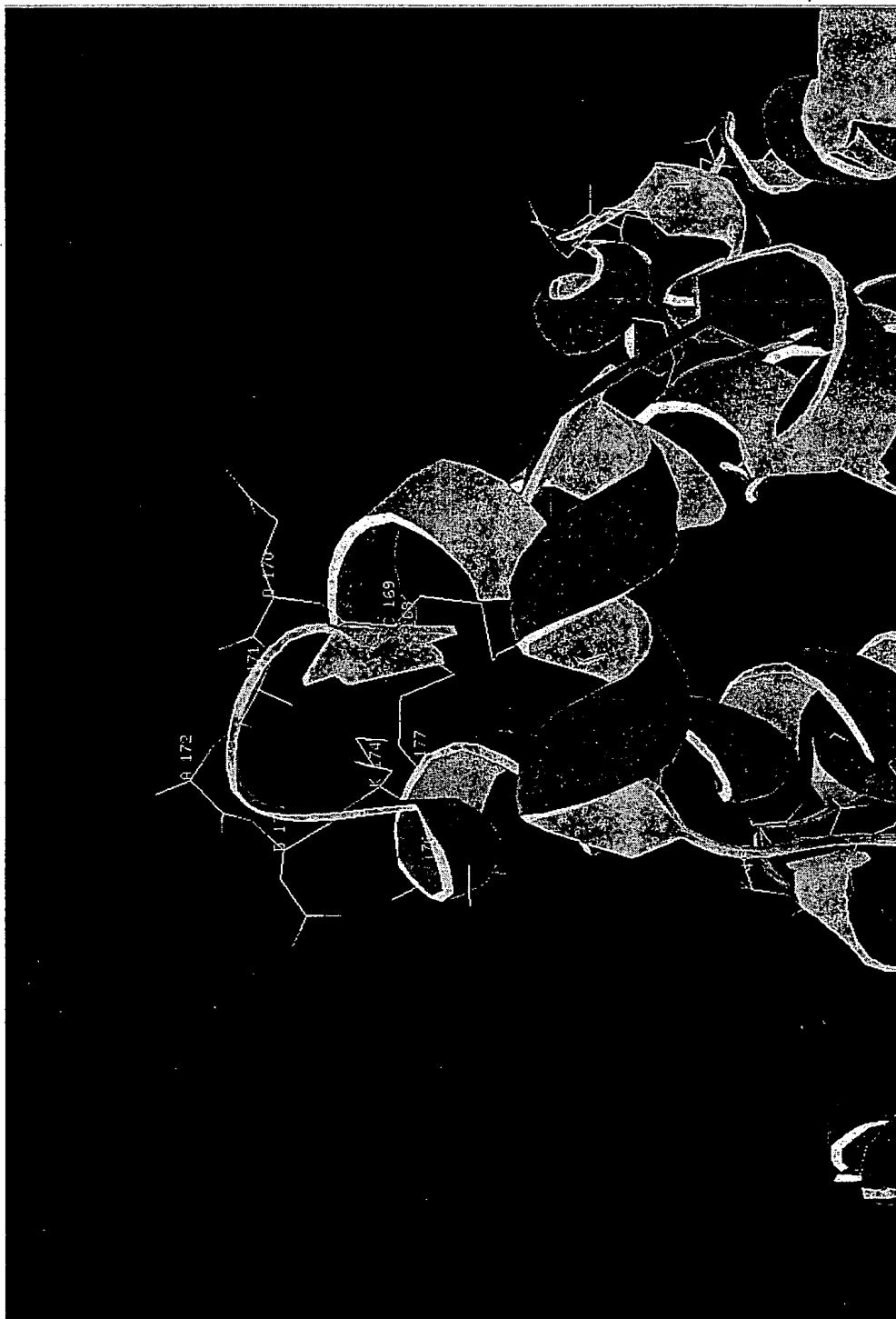


Figure 11



Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176

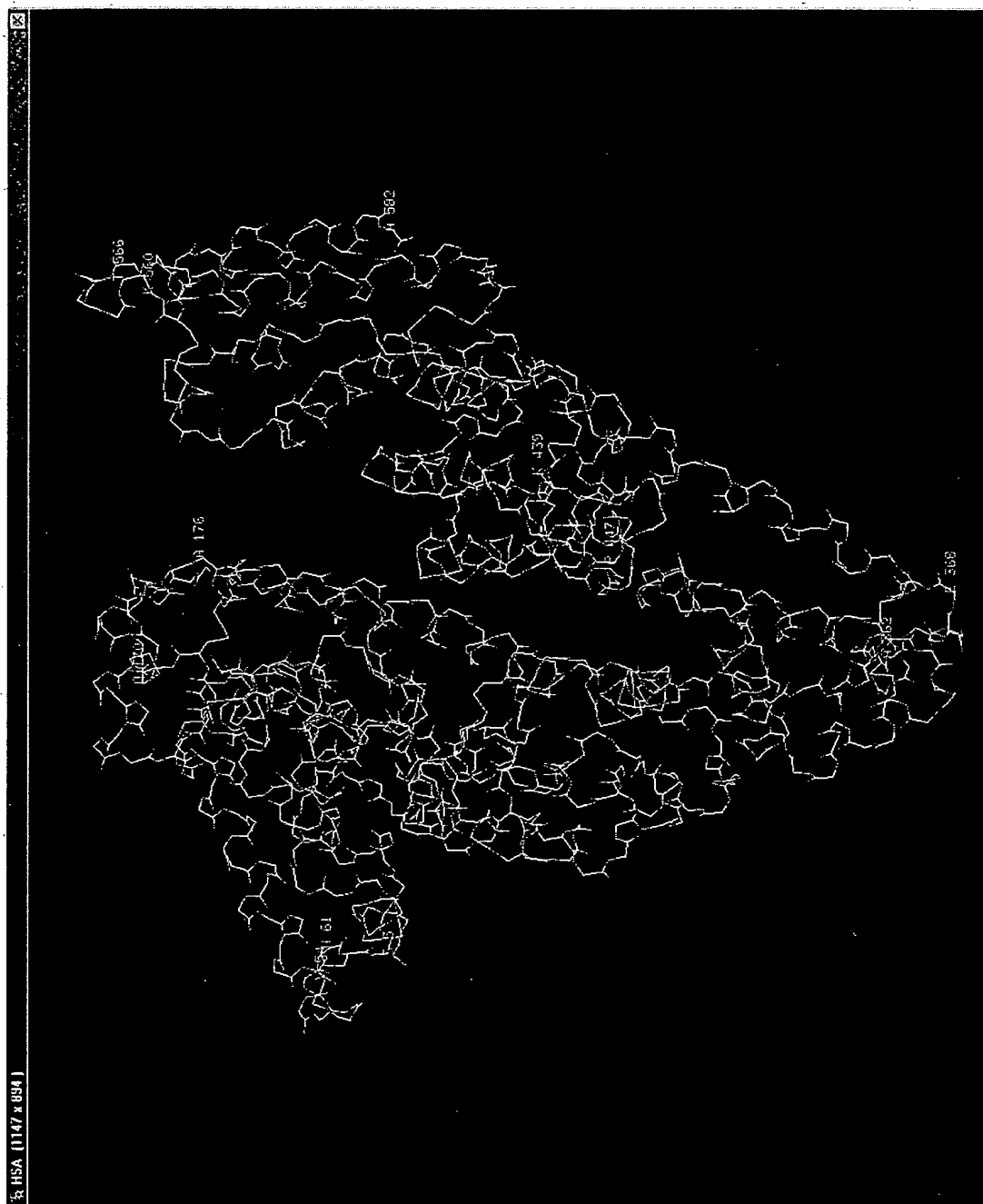


Figure 13: Tertiary Structure of HA

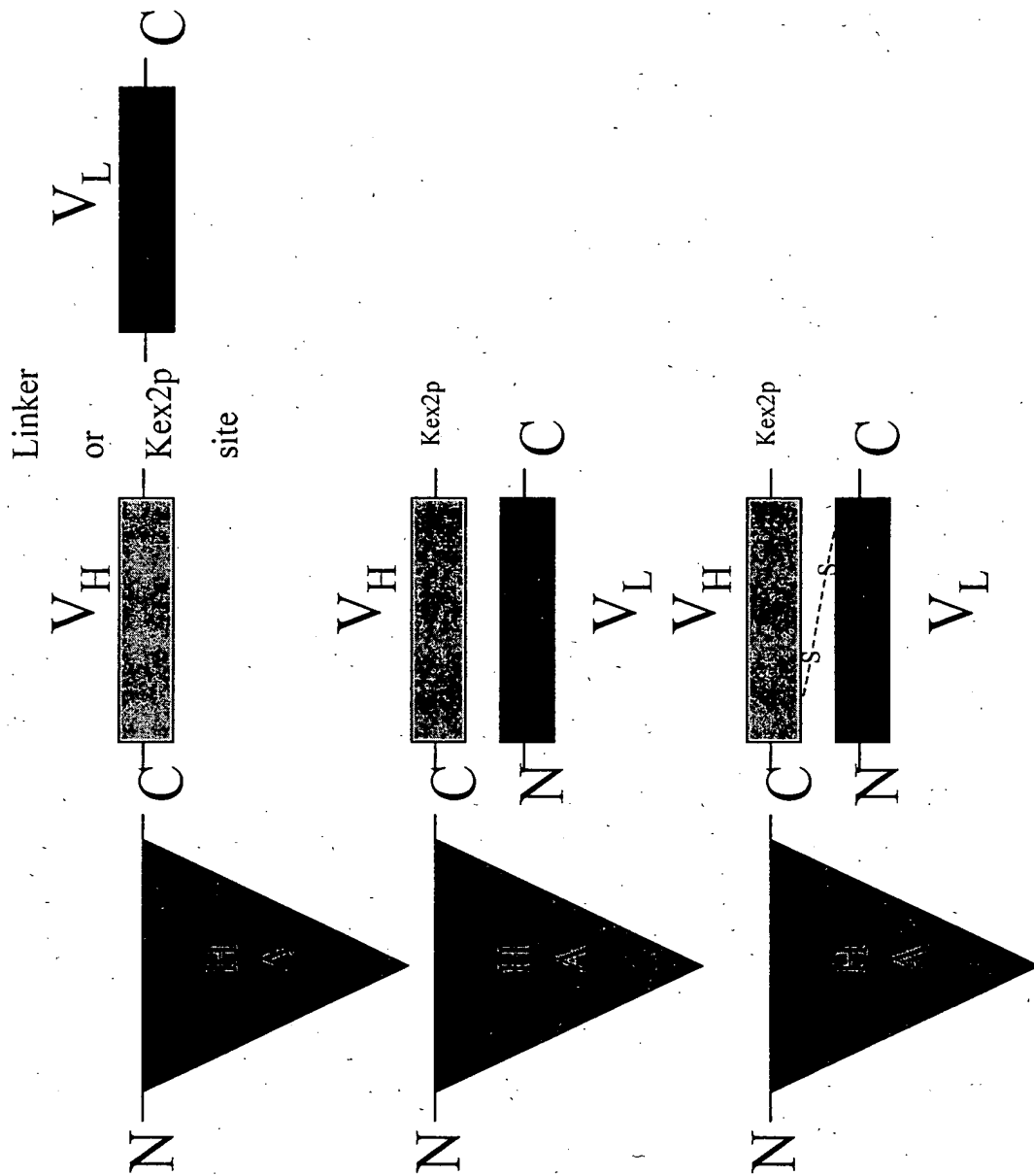


Figure 14: Schematic Diagram of Possible ScFv Fusions.
(Example is of a C-terminal fusion to HA)

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1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA AAT TTC AAA 60
 1 D A H K S E V A H R F K D L G E E N F K 20

 61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
 21 A L V L I A F A Q Y L Q Q C P F E D H V 40

 121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
 41 K L V N E V T E F A K T C V A D E S A E 60

 181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
 61 N C D K S L H T L F G D K L C T V A T L 80

 241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
 81 R E T Y G E M A D C C A K Q E P E R N E 100

 301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
 101 C F L Q H K D D N P N L P R L V R P E V 120

 361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
 121 D V M C T A F H D N E E T F L K K Y L Y 140

 421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
 141 E I A R R H P Y F Y A P E L L F F A K R 160

Figure 15A

481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A S L Q K F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A K Y I C E N Q D S I S S K L K E C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K P L L E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
341 Y S V V L L L R L A K T Y E T T L E K C 360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
361 C A A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
401 Y K F Q N A L L V R Y T K K V P Q V S T 420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

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1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
 481 L V N R R P C F S A L E V D E T Y V P K 500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
 501 E F N A E T F T F H A D I C T L S E K E 520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
 521 R Q I K K Q T A L V E L V K H K P K A T 540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
 541 K E Q L K A A V M D D F A A F V E K C C K 560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
 561 A D D K E T C F A E E G K K L V A A S Q 580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
 581 A A L G L * 585

Figure 15D